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Memoirs of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
Vol. XL. No. 9.

STUDIES FROM THE NEWPORT MARINE LABORATORY.

Communicated by Alexander Agassiz.

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THE DEVELOPMENT OF OSSEOUS FISHES.

II.

THE PRE-EMBRYONIC STAGES OF DEVELOPMENT.

PART SECOND.—THE HISTORY OF THE EGG: CLEAVAGE, FORMATION OF THE PERIBLAST, AND DEVELOPMENT OF THE GERM RING.

BY

ALEXANDER AGASSIZ and C. O. WHITMAN.

WITH ELEVEN PLATES.

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PREFATORY NOTE.

The study of young fishes was one of the early zoölogical interests of Mr. Agassiz, and he had published a number of papers [1] previous to 1883 when the late Prof. C. O. Whitman joined the staff of the Museum of Comparative Zoölogy. During a part of the years 1883–1886, Professor Whitman worked upon the development of some pelagic fishes both at the Newport Laboratory and at the Museum, and some of the results of his studies were published in collaboration with Mr. Agassiz in the Proceedings of the American Academy and in the Memoirs of the Museum.[2]

Though the eleven plates for another part of the Memoirs to be "devoted to cleavage, formation of periblast and the development of the germ ring" were printed so long ago as October, 1885, the accompanying text was evidently not written.

The plates, however, seem of sufficient importance to warrant their publication, and this has been made possible through the kind interest of Prof. R. M. Strong of the University of Mississippi, formerly of the University of Chicago. Professor Strong carried out a thorough search among Professor Whitman's manuscripts for any records upon the embryology of fishes, and has most-kindly written a brief introduction. To Professor Strong and to Mrs. C. O. Whitman, who most willingly aided him, sincere acknowledgements are tendered.

Samuel Henshaw.

[1] The development of flounders.<*Amer. nat.*, December, 1876, **10**, p. 705–708.—On the young stages of some osseous fishes.<*Proc. Amer. acad. arts & sci.*, October, 1877, **13**, p. 117–127, fig. 1–2, pl. 1–2.—On the young stages of bony fishes.<*Loc. cit.*, June, 1878, **14**, p. 1–25, pl. 3–10.— The development of Lepidosteus.<*Loc. cit.*, October, 1878, **14**, p. 65–76, pl. 1–5.— On the young stages of some osseous fishes. Part III.<*Loc. cit.*, July, 1882, **17**, p. 271–303, pl. 1–20.

[2] On the development of some pelagic fish eggs.<*Proc. Amer. acad. arts & sci.*, August, 1884, **20**, p. 23–75, 1 pl.— The development of osseous fishes. I. The pelagic stages of young fishes.<*Mem. M. C. Z.*, September, 1885, **14**, p. 1–56, pl. 1–19.— II. The pre-embryonic stages of development. Part first. The history of the egg from fertilization to cleavage.<*Ibid.*, June, 1889, **14**, p. 1–40, pl. 20–31.

INTRODUCTION.

By R. M. Strong.

During the autumn of 1913, with the aid of Mrs. Whitman, a search was made in the Whitman house, and some fish-embryology records were found. Among them were the descriptions of the accompanying plates. The original drawings for all of the plates except Plate XXXIII were also discovered.

Pencil marks on the original drawings assisted greatly in locating explanatory material, which was found for all of the figures in the eleven plates. It is evident that these are the plates which were mentioned in the footnote (Mem. M. C. Z., 1889, **14**, p. 7). No other manuscript was located but the material available appears clear enough. The pictures are to some extent self explanatory to the embryologist who is familiar with the first paper, and a fairly complete story is told by the records published in this paper. The plates published in the earlier paper contained figures illustrating early cleavage stages but almost no text on cleavage beyond that contained in the "explanations" of plates. Apparently the entire history of the cleavage was planned for the later paper.

The material found appeared in the form of descriptions of drawings (very likely first draft), which were fortunately grouped systematically. The records were not numbered as on the published plates but bore a number which I have placed in parenthesis after the number of the plate as it appears in this publication. The number in parenthesis occurred on the original drawings, in pencil, in almost every case. Thus the description of Fig. 1 in Plate XXXII was numbered 12 in the records left by Professor Whitman, and this number also appears on the original drawing.

It has seemed wise to do little editing, and this little has been done very conservatively. The records are consequently published largely verbatim. Significant changes or additions have been placed in brackets, especially when they have involved the use of considerable discretion. For many valuable suggestions I am indebted to Prof. E. L. Mark.

It should of course be borne in mind that these records and drawings were made a number of years ago without any of the revision that is ordinarily involved in the preparation of a paper for publication. It is not known what changes might have been made in case the authors had carried the material through to publication.

It will be noticed that the records in this publication are remarkably full and readable, as was characteristic of all of Professor Whitman's note making. He was always painstaking and careful in recording his observations, which were made with unusual attention to details. The details were also significant in his note making.

In Fig. 2, Plate XXXII, an embryonic axis is indicated by the terms "anterior," "posterior," "right," and "left." Also in the explanatory material for this plate and for others, references to this orientation occur. Professor Whitman being uncertain about the evidence for the coincidence of the first plane of cleavage with that of bilateral symmetry, suggested the problem to Miss Clapp.[1] She obtained results with toad-fish eggs which caused Professor Whitman to abandon the position, at least for some years. In conversation a few years before his death, he indicated, however, that such an orientation of the early cleavage stages should be looked for in other forms.

This publication is issued with full recognition of the fact that others, notably Kopsch [2] have more or less fully covered the same ground for other fishes. However, no such beautiful and elaborate illustrations of such material have been published, and they should be made available to students of embryology.

The original drawings and manuscript have been deposited in the Museum of Comparative Zoölogy at Cambridge, Mass., where they will be accessible to workers who may have occasion to examine them.

[1] Clapp, Cornelia M. Some points in the development of the Toad-fish (*Batrachus tau*).<*Journ. morph.*, 1891, **5**, p. 494–501, fig. 1–3.— Relation of the axis of the embryo to the first cleavage plane. <Biological lectures from the Marine biological laboratory, Woods Hole. Woods Hole, Mass., 1898. Boston, 1899, p. 139–151, fig. 1–6.

[2] Kopsch, Fr. Die entstehung des dottersackentoblasts und die furchung bei Belone acus.<Internat. monatsschr. anat. physiol., 1901, **18**, p. 43–127, fig. 1–34.— Art, ort und zeit der entstehung des dottersackentoblasts bei verschiedenen knochenfischarten.<Internat. monatsschr. anat. physiol., 1902, **20**, p. 101–124, fig. 1–15.

EXPLANATION OF THE PLATES.

PLATE XXXII

PLATE XXXII.

Fig. 1. (12) Ctenolabrus [*Tautogolabrus adspersus* (Walbaum)] [early 16-cell stage viewed] from below (within). [*bc* = blastocoele or cleavage-cavity], Osmic and Merkel. Cap of cells browned, pellicle stained lightly red. Vacuoles occur along all of the cleavage-lines except *4–4*, *4′–4′*, in which the cleavage is not completed. Along the line *2–2*, especially in the breakage lines of *1–1*, *3–3*, *3′–3′*, the vacuoles form the roof of the cleavage-cavity and are seen to be uncolored, giving the appearance as if the cavity opens above as well as below. But [this is not the case since] there is a line of division running between the vacuoles always. These vacuoles are all on the outer surface of the lines (not on the under side). Very few are seen elsewhere in the cells. The astral arrangement of protoplasm around nuclei is strong, especially in the two pairs of end-cells, which are here plainly larger than the median cells.

The outlines of the middle cells are sharp against the pellicle, of the end-cells obliterated very strongly in region of cleavage-planes (*4–4*, *4′–4′*).

In the corner-cells of the right half of figure we see that, on the upper surface, they abut against the nearest median cell of opposite side. The upper left corner-cell is nearer to the median of opposite side on the under surface than on the dorsal. This is the only case where conditions similar to those in a frog occur.

Notice that the lines of the corner-cells have a tendency to take a radial direction, in harmony with the position of their nuclei.

The cleavage-cavity now covers a little more than the four central daughter cells, the angles being prolonged in the cleavage-lines, sometimes nearly or quite to the periphery. Along line 2–2 the cavity deepens rapidly by sloping sides to the line of vacuoles which form the roof. This cleavage-cavity is found in all eggs of this stage. Only four distinct lenticular spaces seen (dotted in Fig.). Lowering the focus a little we see that the longest ends of the rectangular area dip down beneath the surface, one turning to the right, the other to the left. Just beyond the longest angles are seen two lenticular areas such as are often seen in fresh specimens; these appear to be in the surface. *The nuclei* are also near the surface (upper surface Fig.).

This preparation is from Merkel's fluid, and hence the whole cap is colored dark red, the pellicle being only faintly stained.

Numerous small vacuolar spaces are seen in the spheres, as well as in the pellicle. I do not know if due to reagents. These vacuoles are found in all preparations of the caps.

Fig. 2. (18) Ctenolabrus; 16-cell stage from below. Osm. ¼% 20 m. Merkel. Shows every nucleus in state of division, eight lenticular spaces, the outer boundary of cleavage-cavity (*bc*) and the boundary-lines with vacuoles on the upper surface of cap. The line of junction between the two central cells (*a.b*) is about twice as long on the under as the upper side.

Notice that the general trend of all the nuclei, except two at hind end, is at right angles to the first cleavage-plane. We have here a difference between fore and hind end. The outline of the cap is very sharp everywhere, but the cap is rounded at the margin, so that the pellicle does not join the sharp edge, but does so at a lower level, *i. e.* near the lower (inner) surface of cap. [*Cf.* Plate xxxv, fig. 1, 8th section, top end].

The nuclear figures of four central cells appear shortened because they do not lie horizontally. It is the inner end of these spindles that lie lower (nearer the internal side) than the outer poles.

The twelve marginal cells are stained darker than the four central cells — giving the ring seen in colored drawing.

The achromatic poles of nuclear figures are here quite distinct, they are often convex on the outer side and concave on the inner sides.

1

2

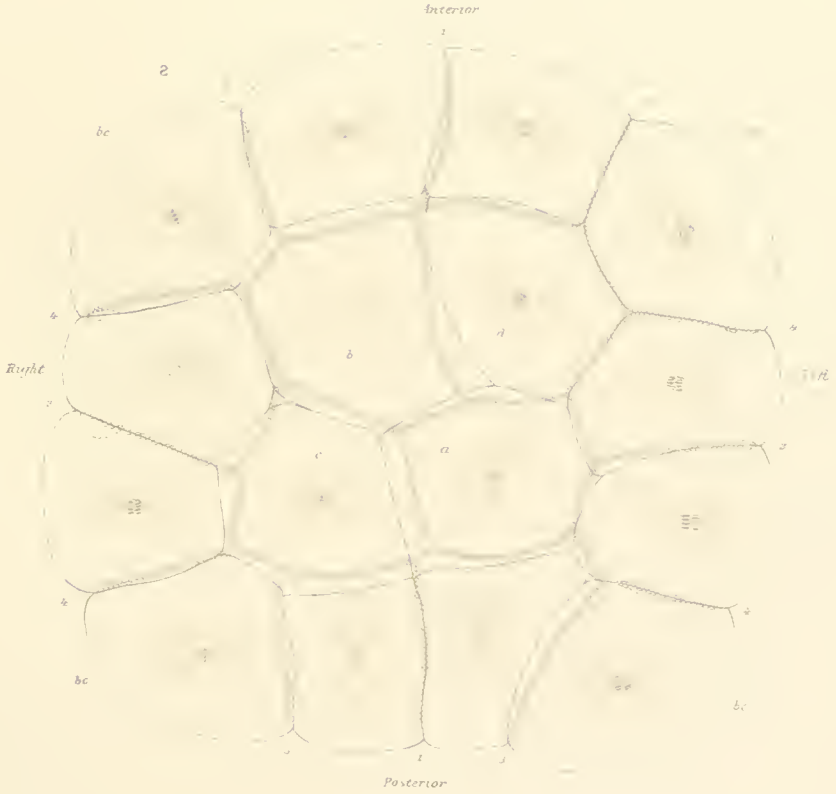


PLATE XXXIII.

Fig. 3. (19) and Fig. 4. (20) Ctenolabrus 32-cell stage in division. [Fig. 3 seen from above, Fig. 4 from below] four central floor-cells dotted. *i. e.* outlined with dotted lines [in Fig. 3]. [In Fig. 3] every nucleus is in state of division. Comparing this with Fig. 4 of the same age nearly, we see that the cap consists of seventeen marginal cells and fifteen central cells. One of the marginal cells, in cases where the cleavage is more symmetrical, as in Fig. 4, takes its place among the central cells, thus making sixteen in the margin and sixteen in center. This cell is *pc* of the right side, which is here plainly in the margin, but its inner face is much narrower than the outer, showing at least a tendency to occupy a position *above* the marginal cells, *i. e.* lapping over the marginal cells as in Fig. 4. Looking at the central cells as seen in Fig. 4, we can say that they are arranged in two double rows, crossing at right angles. The double row (= axial row) lying in axis of future embryo is two cells deep; the transverse row is two cells deep at the center, but one cell deep at the extremities.

Notice that the pair of *pre-central cells* (*pc*) lap over the marginal cells farther than the pair of *retro-central cells* (*r c*). The tendency of one of the pre-central cells to take a marginal position seems to be quite significant, for I find that the majority of caps of this age have seventeen marginal cells: four on each side, four behind, and *five* in front. In most cases the extra marginal cell in front makes the front side more convex than the hind side. This convexity is frequent in the 16-cell stage, but *more* frequent in the 32-cell stage.

In both figures it is the posterior left central (*a*) cells that abut against the diagonally opposite cells (*b*). I do not think this feature is universal.

Of the central cells, four (dotted [Fig. 3, pale letters] a–d) are completely in the floor, only one of them reaching to the upper side (dotted c) and four (*a–d*) are completely above. On the right and left, between the marginal cells and the four central ones are two *intermediate* cells (*i*), which extend from floor to roof, forming part of the upper and lower surface; this appears to be uniform in all cases that I have studied. [In Fig. 4 the lettering is reversed, *a–d* are floor-cells; pale a–d, roof-cells].

The pre-central and retro-central cells always lap, or lie completely above other cells; in Fig. 4 *r c* of the right side is completely shut out from the floor, while *r c* of the left side shows a small part of its surface (*x*) in the floor. The same is true of *pc* of the left side. The retro-central cells take part in forming the floor more often than the pre-central. In Fig. 3, the retro-central cells both show considerable surface in the floor. In perfectly symmetrical caps, we count six cells from side to side, both above and below; while from before to hind side we count five above and four or five below.

Vacuoles: — No intercellular vacuoles could be seen in [the material used for] Figs. 3 and 4, but I have other preparations of the 32-cell stage in which they show beautifully.

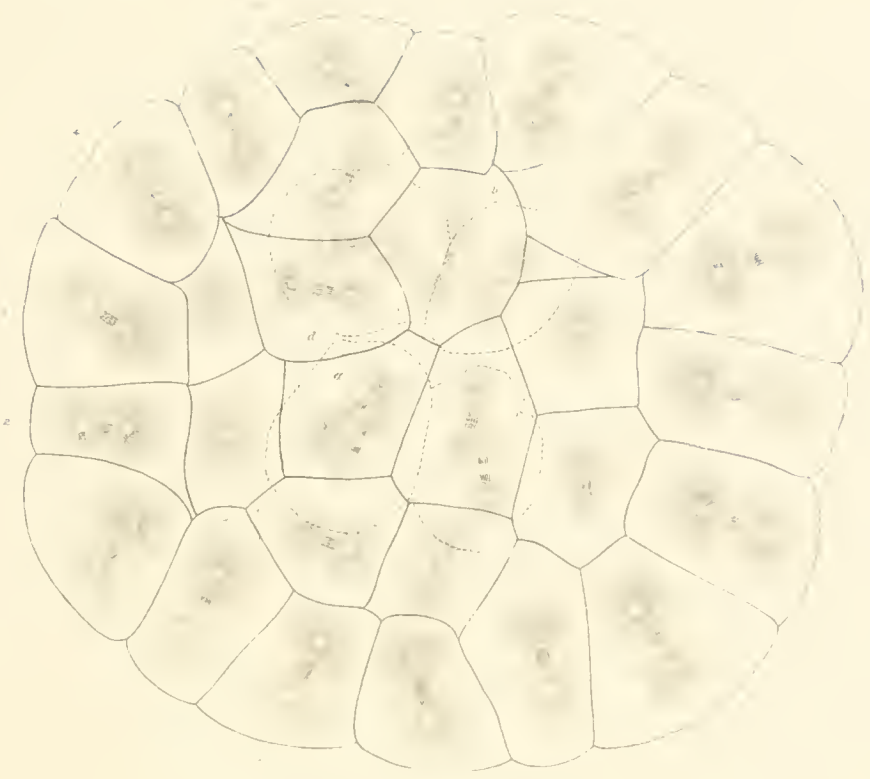
The *dimensions* are usually much wider than long. One is much wider than in Fig. 3.

Nuclei of four lateral marginal cells appear to divide indifferently, tangentially, or radially, those before and behind almost invariably radially.

Nuclei: — As to the nuclei, in Fig. 3, the nuclear figures of the marginal cells have a radial direction. In Fig. 4, they are radial before and behind, but tangential on the sides with one exception on the left side (no. 3). The nuclear figures of the *intermediate* cells are vertical or nearly so. When the nuclear figures of the marginal cells are *radial*, as in Fig. 3, the inner poles lie higher than the outer poles, hence I have shaded the inner poles more heavily. Even when tangential I think one pole may often be higher, perhaps generally so, than the other, so that in any case the division of the marginal cells results in making a ring of cells lying above and overlapping more or less the extreme marginal cells.

Cleavage-cavity: — The limits of the cleavage-cavity (*bc*) are shown feebly in Fig. 4. It is not easily traced in succeeding stages and perhaps may be said to vanish.

Thus a comparison of Figs. 3 and 4 shows that the direction of the nuclear figures may vary considerably and still not obliterate the general plan.



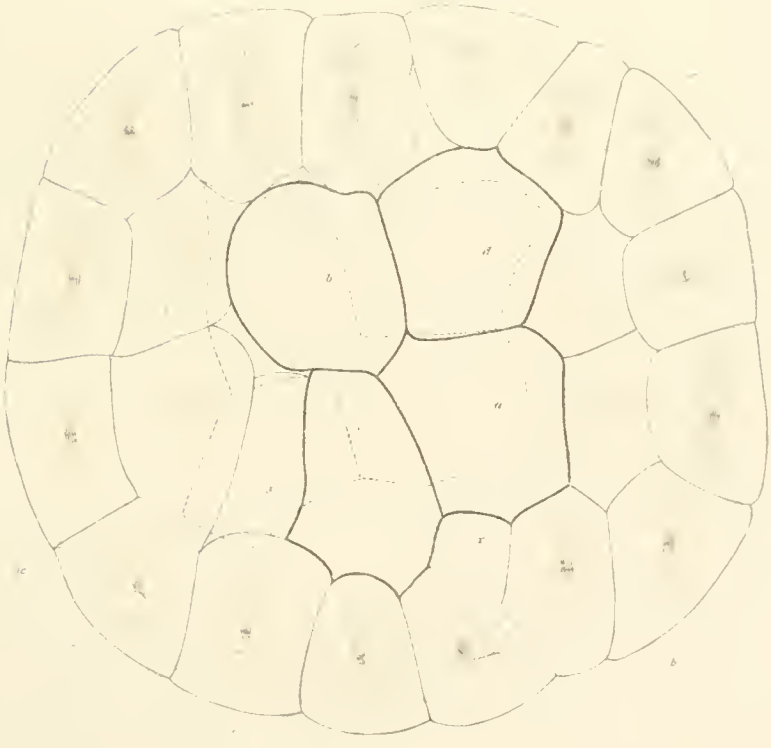


PLATE XXXIV.

PLATE XXXIV.

Figs. 5–6. (21–22) Ctenolabrus 64-cell stage. Fig. 5 from above, Fig. 6 from below. Both are in about the same state of division. In [the preparation used for] Fig. 5 the central cells are stained browner (osmic) than the marginal cells, and their distinction was only somewhat less noticeable in [the view shown in] Fig. 6. For this reason and for convenience of analysis, I have given heavy outlines to the central cells, and dotted outlines to the cells seen through the exposed surface.

I see no reason to doubt that the lettering in Fig. 6 is precisely as it should be in relation to the lettering of Fig. 4 of the previous stage. In Fig. 5 there is no obscurity except with regard to which ones of the marginal cells represent *pc* and *rc*. However as *pc* of the left side is certainly identified, it seems probable that [the numeral] *5* [between *2* and *3* of the anterior marginal cells], represents *pc* of the right side. If these two are correctly identified then there can be but little doubt in regard to *rc–rc*.

In both figures, assuming the division completed, we have thirty-six cells in the margin and twenty-eight in the center. Of the thirty-six cells, eighteen lie above (obliquely) the other eighteen, the eighteen of the upper surface, lying for the most part nearer the center, while the eighteen of the lower layer stretch away farthest from the center, extending out so as to present one half or more of the upper surface to view. Although Fig. 6 exhibits considerable variations from the radial direction of the nuclear figures which prevails in Fig. 5, still the direction is such that in the majority of cases the inner pole of the figure lies nearest the upper surface, so that the division is not horizontal, but *oblique*. The pole nearest the observer is more strongly shaded in both cases.

It is very interesting to note that the outer poles in many cases lie extremely near the margin, and, particularly in Fig. 6, one can see that the marginal daughter nuclei lie *very* near the under surface — yolk surface — of the cap. Also it is interesting to note that the marginal cells often pass into the pelliele with diffuse or blended outline.

May not the marginal cells already be considered as endoderm and the upper marginal as mesoderm? It is quite certain that all the central cells represent ectoderm.

It is important to note that in all these stages, the two intermediate cells (*i–i*) maintain uniform relations and always divide horizontally in passing to 64-cell stage. This confirms the view that the embryonic axis coincides with first plane of cleavage.

Again a portion of *rc* (*5* in Fig. 6) lies in the floor while the marginal portion lies mainly above the cells *3* and *4*. The left cell *rc* also divides obliquely, one half showing in the floor. In Fig. 5 right *rc* lies wholly in roof, left *rc* lies wholly in margin.

In Fig. 5 left *pc* touches the floor at its right end, while right *pc* lies in margin. In Fig. 6 right *pc* lies mainly above *2* and *3* and left *pc* divides obliquely, one half showing in the floor.

The nuclei when seen frontally, look like a line of large elongated granules. Viewed from the pole they generally present the form of a *ring* of granules. The achromatic poles are sometimes very plain. Some nuclear rays reach beyond the outline of marginal cells, into the pellicle. [Fig. 6, no. 1 in anterior row of cells].

Vacuoles are seen only in the outlines of the roof-cells — nowhere in the floor-cells; they are very neat in both preparations but have been omitted from the figures.

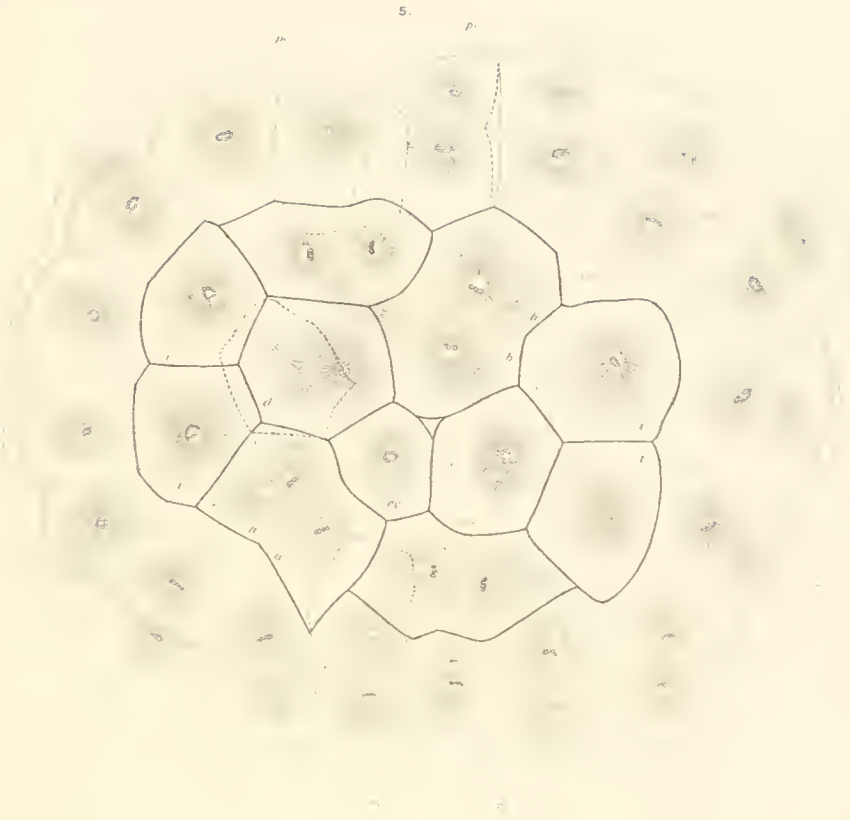
The cleavage-cavity cannot be traced with certainty; possibly it no longer exists.

In a 64-cell stage of same set, but 30 min. later, I find two of the marginal cells, at the fore or hind end of the future axis that have taken on the form and appearance of true endoderm cells.

Most of the preparations on the slide used for Figs. 5 and 6 are in the 32-cell stage. One of these 32-cell stages had a remarkable symmetry.

There were sixteen cells in the periphery and sixteen in the center. The retro-central and pre-central cells did not lap but stood abreast, like the pairs of intermediate cells, reaching from top to bottom of cap. Thus the cap was two cells deep only in the four central cells.

Endoderm: — Although I believe the 64-cell stage practically settles the endoderm and that some of the cells in this stage very often assume the endodermal condition, I think that they resume their outlines and keep the marginal position in the cap for some time later. Two hours later than Figs. 5 and 6, I find the endodermal wreath more or less conspicuous in all, but in some the wreath has not yet included more than a part of the marginal cells.



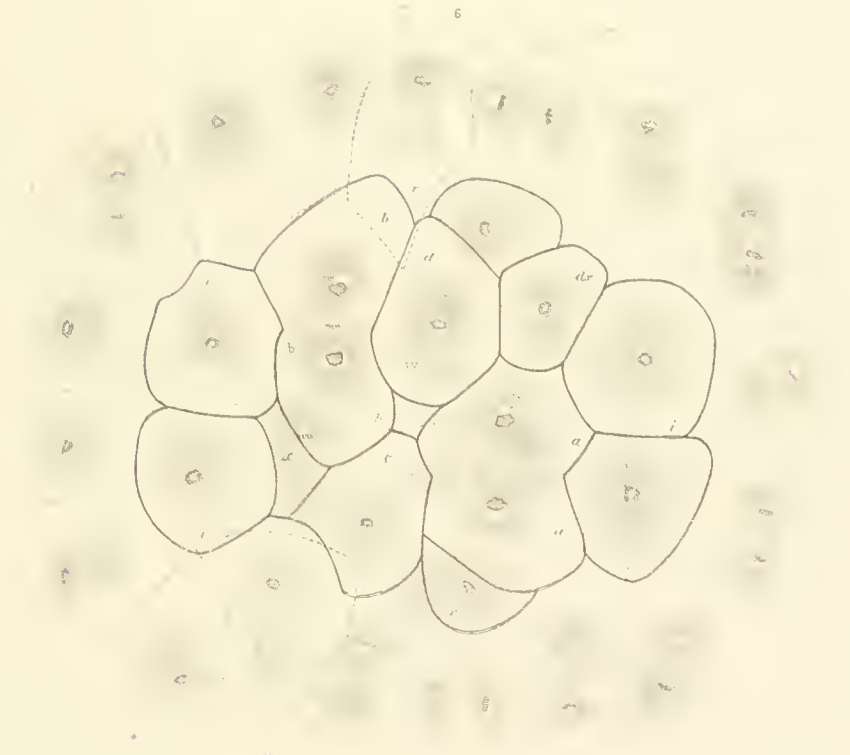


PLATE XXXV.

PLATE XXXV.

Fig. 1. (71) Ctenolabrus. [16–32-cell stage. Somewhat older than stages shown in Plate XXXII, Fig. 2]. 9 drawings of a single cap (successive) × 280. [*bc* = blastocoele; *pb* = periblast]. Corresponds to two hours 35 minutes after fecundation.

The second section has not reached nuclei but has the clear areas.

Periblast: — The *periblast* (subgerminal plate) is seen between the cells in the angles formed by the adjacent cells. It is quite thick (about .004 mm.). It looks like a thick dense membrane, is very sharp in outline, and stops abruptly in the angles of the cells. It is thus continuous with the lower surface of the cells. There is a strong contrast between it and the protoplasm. The latter is granular but the periblast is more homogeneous and membrane like. It is quite as strongly stained with carmine as the protoplasm. Very small open triangular spaces are seen above the periblastic parts.

The third section shows larger spaces in angles of cells and reveals more extensive position of periblast. One pole of three nuclei is seen. The periblast is a little less thick.

The fourth section shows the other poles of two [of these three] cells. The periblast terminates in the angles of two peripheral cells, and is entirely free from the central ones, except perhaps the right central, under which no distinct membrane can be seen but a light colored substance extends beneath this cell connecting the periblast. The periblast would seem to form by growth from the superficial yolk. This explains why it is often wanting near the center of floor, and often appears continuous with a superficial lighter colored layer of the yolk, as seen in section eight.

The eighth section cuts two marginal and two central cells. (*The four central cells are blacker than the marginal ones* as was seen both before and after sectioning). *Here the plane of division is marked. In the central cells observe the inclination.* Notice the nuclei; they are in edge of light areas, and a poorly defined small area, about size of nucleus or a little larger, is seen in each case on the outer pole of the nucleus.

The periblast is much thinner, becomes less dense, lighter, and poorly defined under central cells. Yolk [is indicated] below these cells to show that the forming periblast merges into it.

The ninth section = same cells.

The tenth section = right cell, at middle, in passing to next cell.

The twelfth section corresponds to section eight.

The nineteenth section corresponds to section four.

The periblast forms a continuous sheet in sections seventeen, eighteen, and nineteen, but is interrupted in the twentieth. It is very thin centrally in section seventeen, plainer in eighteen, plainest in nineteen.

Fig. 2. (83) 32-cell [stage section; *bc* = blastocoele]. The central cells are browned more than the marginal, the marginal are redder than the central.

The interesting fact is that the lower central cells are also more brown, just like the upper central cells, thus appearing to have the same value.

Fig. 3. (84) Transverse section, near middle [*pb* = periblast]. On the right [lower end of figure] is seen a nucleus in the periblastic swelling or *rim*. Perenyi swells the material a little and renders it easier to trace the periblast. The periblast stretches across in a very thin evanescent layer. Along the middle, it is not seen, except between two cells, where it appears to be continuous with them. Cap two layers deep in central part, but these central cells are all browner than the marginal in eggs treated with osmic and Merkel.

1.
2
3

PLATE XXXVI.

Fig. 1. (4) Ctenolabrus. *One hour after 32-cell stage.* [*pb* = periblast]. Out of a large number of caps (20) of this stage, this one alone shows decided evidence of the first beginning of the endoderm. The cap is two to three and four cells deep in the central portion, becoming two and then one cell thick at the edge.

The upper layer of cells is not dotted, and the nuclei are colored, for sake of distinction, more deeply than those of underlying cells. There are considerable intercellular spaces as seen in undotted portions, which are probably due to the cells having been slightly disturbed, or to action of reagents. Numerous vacuole-like spaces (round) varying from size of nucleus to much smaller, are seen prevailingly in the ectoderm.

Histological evidence of a differentiation of endoderm from edge of cap: —

1. The edge-cells are stained with carmine — not browned and the nuclei are all stained with carmine, with only faint traces of browning; while the remaining cells are more or less deeply browned and the nuclei also more browned, and less brilliant than those of endoderm.

2. The radial arrangement of protoplasm is much more accentuated in the endoderm-cells than in the ectoderm, and the cells are rather more coarsely granular. We have here the best kind of evidence that the endoderm arises *late*, from the edge of the cap — thus from what may be regarded as the vegetative portion of cap. The endoderm is in process of differentiation, only certain cells having advanced so far as to be entirely outside the cap and with the characteristic absence of distinct cell-boundaries.

Four cells (*en 1, 2, 3, 4*) have advanced to the syncytial stage — representing four free nuclei in the thickened rim of the pellicle (*pb*), around which the radial lines are very clear and strong. Most of the nuclei appear to be in a condition that precedes the formation of [mitotic] figures. These four cells are wholly within the pellicle, and hence beneath the level of the outer ectoderm-cells.

The cells marked *en* are cells destined to become like *en 1–4*, but have not lost completely, except in limited portions, their definite boundaries. Many of them are not sharply defined *against each other*, or *against* the *pelliele*, with which they are continuous.

ec en 1 is a cell still in the outer rim of ectoderm, but it loses the sharp outline and blends with the pellicle and with the endoderm-cell at its right.

ec en 2 is another similar cell, which still preserves its outline, but which lies in same level as *en 1*.

ec en 3 still holds its position in the ring of cells *en*, but it is not more than very faintly and imperfectly delimited from the pellicle.

We have here, then, the outer ring of cells of cap in process of becoming fused with the pellicle, only four cells of which have become undoubted syncytial cells.

Fig. 2. (16) About one hour after 32-cell stage, from above, treated in same manner and on same slide. Here the cells are about half the size of those in Fig. 3, indicating that a single division has occurred.

Here are seen the same coarsely granular marginal cells in floor of cap, but we see that they have divided tangentially, so that we have here two cells where we before had only one. The outer row of cells is more coarsely granular than the inner row, and their nuclei are at a little distance from the dark margin of the cap proper.

Now I am inclined to take this outer row of cells as representing the first formed endoderm-cells; still it is possible that the inner row is also endodermic. If the outer row alone is endoderm, then in Fig. 3, we should have to say that the endoderm has not yet separated from cap, but simply appears as a more coarsely granular part of the marginal cells. In this case Fig. 2 would correspond in age to Fig. 1, which has also about twenty cells in the marginal endoderm.

Fig. 3. (15) Ctenolabrus. Portion of cap about 30 minutes after 32-cell stage. From below. Stained with osmic only. [*ec* = ectoderm; *en* = endoderm; *pb* = periblast].

There are about twenty cells in the periphery of the cap, forming the marginal cells of its floor and projecting a little beyond the smoothly outlined superficial cells. These cells are everywhere sharply defined except against the pellicle. Their boundary line against the pellicle is tolerably distinct but not smooth. It is more or less ragged as if the delimitation was not complete.

An important feature of these cells, in contrast with the finely granular cells elsewhere, is their coarsely granular nature. They are more and more coarsely granular as we pass from the inner to the outer margin. The outer half of these cells is thinnest and more coarsely granular than the inner half.

Notice also that the nuclei of these cells are in the first stages of division; *i. e.* they are elongated radially and are faintly striated with a darker dotted line in the middle zone. The achromatic spindle and stellate rays are not to be made out, the preparation not being favorable to making out fine details of nuclear structure.

These cells fit into the other floor-cells which are shrunken away from them at this point.

Fig. 4. (87) One hour 30 minutes after 32-cell [stage]. [*pb* = periblast]. Section near middle. Here the marginal cells are very distinct, but owing to method of treatment the subgerminal plate is very indistinct. Cap two cells deep at margin (sometimes two sometimes only one), three cells deep in middle. All the central cells from top to bottom are blackened alike.

Fig. 5. (85) Transverse section near middle of 64-cell [stage], (30 minutes after 32-cell) Os and CrO_3 3 days.

The marginal cells are lighter and redder than the central, the latter being slightly more tinged with osmic. The cells are closely packed and the subgerminal plate everywhere in contact with cells except at one point where it is broken. This is probably due to the contraction caused by the chromic acid.

Fig. 6. (86) One hour after 32-cell = fig. 1 in age). Transverse [section] near middle. (Os and Mk. 3 days). [*bc* = blastocoele; *pb* = periblast].

The periblast is continuous under cap in most places. The marginal cells are distinct in color.

In all caps of this age, I find the central cells smaller than marginal and from two to three cells deep; the marginal are from one to two deep.

en 4
pb
en 3
ecen 1
ec
en 2
en 1
pb
ecen 2
ecen 3
en
pb
2
en
pb
3.
ec
pb
4
5
pb
6
pb
pb
pb

PLATE XXXVII.

PLATE XXXVII.

Fig. 1. (8) Ctenolabrus from inner surface. Os $\frac{1}{5}$% 15 minutes, Merk. 3 days. 2 hours after 32-cell stage.

In the wreath are about twenty-seven cells. Protoplasm radiate around some, no nuclei in division. Cap = two to four cells deep; smaller in central, larger in periphery. Round cells are seen in the central portion.

en 1, 2, 3 = delimited.

en 4 not delimited against pellicle.

en = syncytial endoderm-cells.

Endoderm colored red, cap brown.

For distinction, nuclei of *en* are red, nuclei of cap brown (Indian red).

Most of *en* lie under, not outside, the margin of the cap. In a number of cases the nuclei are half under cap, half exposed. [*pb* = periblast].

Fig. 2. (88) Two hours after 32-cell [stage]. 42 sections .005 mm. thick. [*pb* = periblast].

The first section cuts edge of cap. Dividing line between marginal cells does not cut quite through the periblast.

The twenty-first section through middle. Marginal cells small, but well colored red, while the central cells are all brown.

The twenty-fourth section shows an inner cell that is reddish, but less so than the marginal cell.

The twenty-seventh section shows no marginal cell.

The thirtieth section shows red cell above the marginal cell.

These facts are to be explained by supposing that the marginal cells are constantly dividing and adding cells to the cap, which soon after division become like the other cells of the cap, taking brown color from osmic acid.

At this stage the subgerminal plate is pretty complete everywhere.

It is the upper portions of the marginal cells that are constantly cut off and added to the cap, until at length the basal portion ceases to have any connection, and its nuclei spread in all directions.

Fig. 3. (9) Ctenolabrus. Two hours after 32-cell stage. [*pb* = periblast]. A portion of cap from above. The endodermal wreath is here very distinct and contrasts with the ectoderm everywhere in the staining. There are ten pairs of cells and an older one, en^1. Three of the pairs [en^1, en^2, en^{10}] are tangential and the rest are oblique or radially placed. In *en 1* the division is so nearly completed that only the most faint traces of interzonal filaments are discernible. The right hand cell of the pair is in plane above the left, and [is] more sharply defined but colored the same.

en 2 shows last end of division with filaments still visible. A light line between cells indicates plane of division which is not completed. Both cells continuous with pellicle (*pb*).

en 3, [The number does not appear on the plates, but the cells are evidently those between *en 2* and *en 4*]. Division completed, inner cell highest, and best defined, but colored lighter than rest of ectoderm.

en 4, inner cell also highest but below the highest of pair *en 3*. Division completed and outline clear in both.

en 5, same as in 4.

en 6, both in same plane — faint traces of spindle.

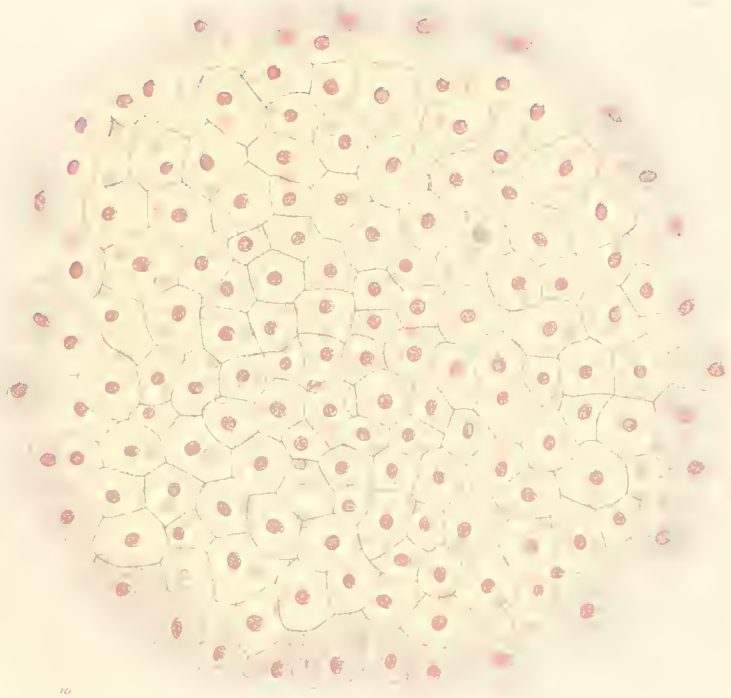
en 7, division completed.

en 8, faint spindle-cells not sharply outlined except against the floor-cells of cap.

en 9, outlined inner cell lighter than ectoderm and appears to be paired with the outer longer cell.

en 10, division not completed, pretty well defined but flowing into pellicle.

All the inner cells of the pairs, except in *en 3*, lie in the floor of the cap, and in most cases abut against surrounding cells. The paired nature of these cells is most evident. Most of the inner cells are covered by ectoderm but wherever they are uncovered, they are colored like endoderm.



2

3

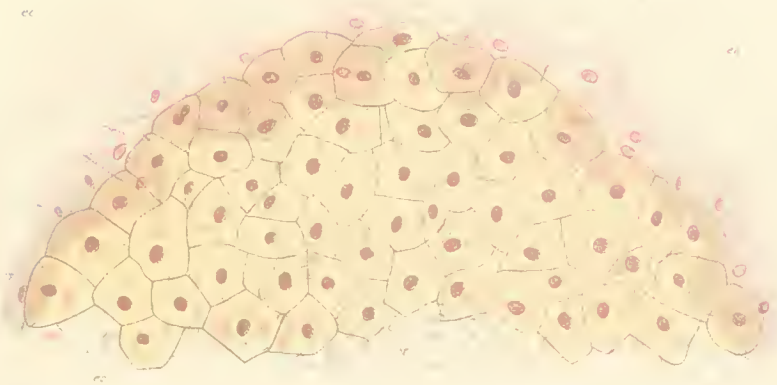


PLATE XXXVIII.

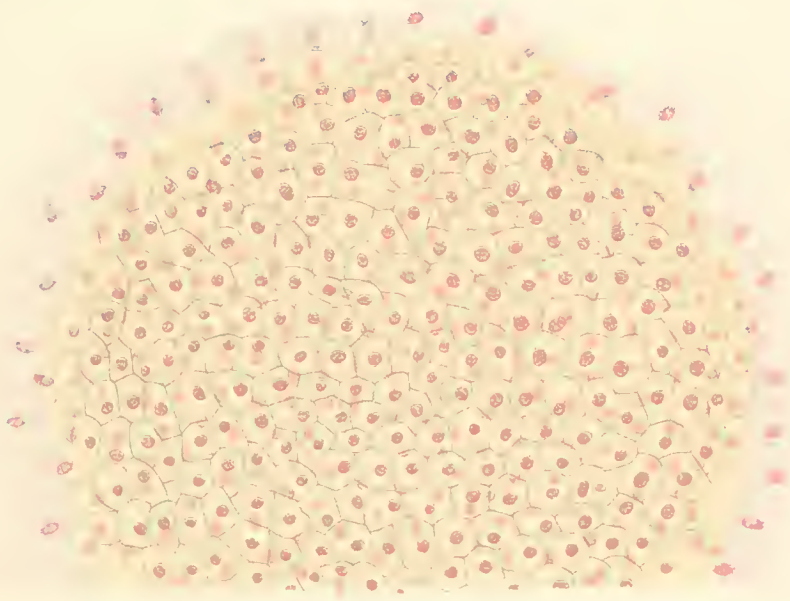
PLATE XXXVIII.

Fig. 1. (2) Cap of Ctenolabrus. [*pb* = periblast]. Osmic 15m. ($\frac{1}{3}$%), Merkel 3ds. Same age as [in Fig.] 2, but not *quite* so far advanced, *i. e.* the nuclei of [marginal] row are somewhat elongated, and in a few cases faint asters are seen at the poles of the nuclei, but no striation is visible in the nucleus.

The entoblastic wreath is characteristically granular and thins out rather abruptly into the pellicle, just beyond the nuclei. The cells on both surfaces of cap were distinct. The cell-boundaries around these [wreath] nuclei were visible here and there, but very faint as shown in figure. Now and then a nucleus of a second inner row — just under edge of blastoderm — is seen. Here also the nuclei are mostly elongated in a radial direction.

Color: — Wreath and pellicle colored slightly with carmine, but the cap browned with osmic. The contrast between *granular pink* wreath and light brown cap is striking here as in all cases thus treated.

Fig. 2. (1) Cap of Ctenolabrus. [*pb* = periblast]. Seen from outer surface three hours after the 32-cell stage is reached. To the cap adheres the entoblastic wreath, which is thickest at the edge of cap, gradually thinning out towards the periphery, which is ragged in consequence of being broken from the pellicle. This wreath is stained faintly with carmine (borax alcohol) while the cap is browned by osmic acid and not stained by carmine. The wreath reaches under the cap for a short distance but not to the central parts of cap. There is a single row of nuclei, radially placed (some tangential and oblique), and *all* in process of division, mostly showing a well-marked nuclear plate in different stages of division, and asters (rather faint). The cell outlines about the nuclei are not visible, except in a few instances. Most of the nuclei of the ectoderm are round; one is seen in division, and beneath it is a nucleus of the endoderm-wreath in division. Although there is, broadly speaking, only one row of nuclei visible, an inner row makes itself apparent in several places and now and then a nucleus (dividing) may be seen just under the edge of the cap.



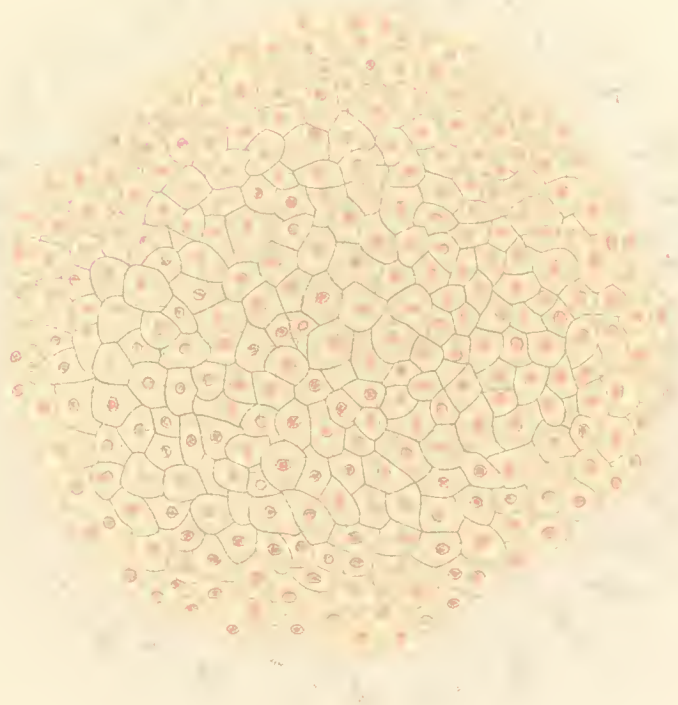


PLATE XXXIX.

PLATE XXXIX.

Fig. 1. (3) Ctenolabrus, under (inner) surface. Treated as Figs. 1 and 2, [Plate XXXVIII], four hours after 32-cell stage. One hour after Figs. 1 and 2 [Plate XXXVIII]. In this cap the nuclei of the endoderm are strongly colored and very well defined. One can see by the nuclei that the endoderm extends for only a short distance under the cap. The majority of the nuclei are outside the cap.

Fig. 2. (89) [Seven sections]. Three hours after 32-cell stage. [*bc* = blastocoele; *pb* = periblast]. (Os, Mk. 3ds).

Second section shows only one or two outlined cells, the outlines being indistinctly marked.

The third section shows two well-outlined cells; the right is sharply marked inferiorly. There is a partial outline of a dark cell of the cap.

The fourth section shows the first appearance of the sub-germinal plate. All the red cells are shaded by dotting.

The sixth section shows the subgerminal plate thinner.

The sixteenth section shows on the left a red cell that looks as if it was to enter into cap.

The twenty-sixth section is near middle. Here sometimes no nucleus is seen in periblast, at other times one or even two. Subgerminal plate very thin, wavy in outlines as in section sixteen.

The twenty-eighth section shows an inner cell in process of splitting off, possibly destined to become one of the cap cells.

The thirty-third section showed a similar case.

I think it is possible that cells are still added to the cap, but that this process is nearly concluded, so that the periblast as a cell layer may be now considered established.

Fig. 3. (90) [Later stage. *bc* = blastocoele; *ep* = epidermis; *pb* = periblast]. Section near middle. The periblast is very thin, vanishing or nearly so near the center of field. Epidermis well marked off.

On left is a single periblast cell that looks as if it was a cap-cell. The fact that the periblast becomes so very thin indicates that cells are added to the cap up to about the time the ring begins to form.

The nuclei of the periblast are still confined to the thickening beneath the margin of cap; on the left [*i. e.* lower end of figure] a single nucleus is somewhat advanced from the margin towards the center, but this is exceptional in these sections.

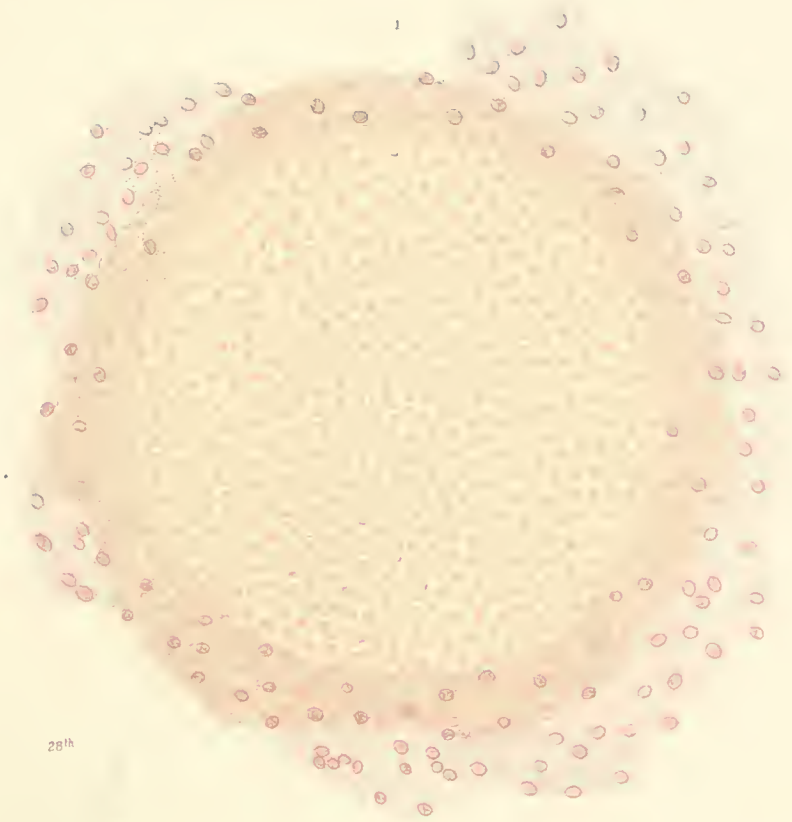

1
28th
2
4th
3rd
pb

PLATE XL.

PLATE XL.

Fig. 1. (92) *P.*[*aralichthys*] *oblongus.* [*bc* = blastocoele; *ep* = epidermis; *pb* = periblast]. 61 sections. This figure is the twenty-fifth [of sixty-five sections] and is like the middle ones. The periblast is thin but extends farther under the cap than in Fig. 2. Near the center the layer fades out and is not recognizable, but four nuclei are very distinct, showing that the layer exists though thin and perhaps not fully differentiated. The nuclei extend under the entire cap although the ring has scarcely begun.

This shows that the periblast has already begun to expand as an independent layer and has therefore probably ceased to contribute directly to the cap.

Fig. 2. (91) *P. oblongus.* [*bc* = blastocoele; *ep* = epidermis; *pb* = periblast]. Three hours after 32-cell stage. Four of sixty nearly longitudinal sections. Left end = embryonic region.

The second section does not reach cap; third touches cap.

The fourth section shows some of cap, and periblast thinner at middle.

The eleventh section shows periblast still thinner in middle. Nuclei are more numerous at left end all through these sections. I think the periblastic nuclei are more numerous under region of embryo than elsewhere.

On the seventeenth section the periblast is scarcely visible at center beneath cap. On the sixteenth two nuclei were found near the middle of the subgerminal plate, although the plate is scarcely traceable.

One nucleus was seen near middle of the nineteenth section and one in twenty-fourth. The periblast is somewhat less in bulk on the middle sections than on the thirty-eighth which I have drawn. Periblast is here about same in quantity as in Fig. 2, Plate XXXIX. (Three hours after 32-cell).

pb
2
4th
bc
pb
bc
11th
pb
ep
bc
38th
pb

PLATE XLI

PLATE XLI.

Fig. 1. (96) (*P. oblongus*) from above. Very early stage of ring. [*pb* = periblast]. The ring has just become well defined everywhere and the embryonic fold is seen somewhat bulging centripetally. The ring is nearly even in width everywhere, except for being a little wider as it enters the embryonic region.

Width of ring = .02 mm.; in embryonic region = .04 mm.

Cap almost perfectly circular. Diam. of cap = .55 mm.

The *thick wall* (wreath) of periblast is not yet fully covered so that about one row of nuclei is seen all around. The number of nuclei could not be accurately determined, so they were put in at random guided by what was known of other cases. The outlines of only superficial cells (epiblast) are seen, but nuclei of deeper cells are given. The epiblast is seen in profile along the margin. The posterior half of the cap is thicker than the anterior, and this is shown by heavier dots and lines. The cap should appear convex — but the lithographer could [not] be instructed to shade it properly.

The posterior [lower half of figure] half is the embryonic area, the anterior [upper] the pre-embryonic area.

The ring is now only .02 mm. [in width], later it becomes .12–.15 mm. in width.

Fig. 2. (97) [Section of] cap before appearance of ring, cleavage cavity shallow.

Fig. 3. (98) [Section] just before ring. [*bc* = blastocoele; *pb* = periblast]. Embryonic area thicker than pre-embryonic area. (Large yolk cells supplied).

Fig. 4. (99) [Section. *bc* = blastocoele; *pb* = periblast]. Blastoderm thinned out much in anterior half, where for some distance the lower layer is only one cell deep. Towards the anterior edge the under layer thickens.

PLATE XLII.

Fig. 1. (94) *P. oblongus.* Sections drawn at intervals. Young ring stage cut transversely into sixty-three sections (.0075 mm.). [*bc* = blastocoele; *ep* = epidermis; *pb* = periblast].

Diagram 12 = longitudinal median section, constructed from the transverse sections. .0075 mm. × 280 equals apparent thickness of each section. This amounts to 2.1 mm. 2.1 × 63 gives apparent length of the cap, which is 132.3 mm. Measuring off 132.3 mm., I divided it into sixty-three equal parts, and then by vertical measurements of each section constructed the diagram. Thickness of upper layer in the sections. (Apparent of magnified 280).

8th section	= 15 mm.	27th section	= 11.5 mm.
10 " "	= 16 "	28 " "	= 11 "
12 " "	= 15 "	29 " "	= 10 "
13 " "	= 15 "	30 " "	= 10 "
14 " "	= 14 "	31st "	= 10 "
15 " "	= 15 "	32d "	= 10 "
16 " "	= 13 "	33rd "	= 10 "
17 " "	= 14 "	34th "	= 9 "
18 " "	= 13 "	35 " "	= 8 "
19 " "	= 14 "	36–50th "	= 8 "
20 " "	= 14 "	53d "	= 10 "
21st "	= 13 "	54th "	= 9 5 "
22 d "	= 13 "	55 " "	= 9 "
23rd "	= 13 "	56 " "	= 10 "
24th "	= 12 "	59 " "	= 10 "
25 " "	= 12 "	60 " "	= 8 "
26 " "	= 13 "	61st "	= 8 "

The second section takes in many epiblast cells, which are seen partly in surface owing to the convexity of the cap near margin.

Nearly all the cells are in some stage of division throughout all the sections.

Notice that the nuclei of the periblast are few in section two, more in three, most in five and are fewer towards the middle of cap (37th). They are more numerous beneath the embryonic fold than elsewhere under the ring.

The *lower layer* becomes distinct only in section five, where we see not only a line of division, but notice that the nuclei retreat from this line somewhat.

The lower layer is thickest (3–4 cells deep) in the embryonic fold; elsewhere the ring where it is well begun is 1–2 cells deep. The ring is seen as a true *infolding* in the thirty-second section and especially in the thirty-fifth. In some points the ring is scarcely begun, *i. e.* there is no distinct ring. The axial portion of the ring ceases with the tenth section, being thus less than $\frac{1}{6}$ of the entire length of the cap.

The *upper layer* is thickest over the embryonic apical fold, being here from 4–6-cells deep. This thick area thins off gradually beyond the twenty-fifth section (*i. e.* between twenty-fifth and thirty-fifth). From the thirty-fifth onward the diminution in thickening continues but in a much less marked degree, dwindling down to about half the thickness of the embryonic fold.

The thickness of the upper layer is nearly uniform in all transverse sections from margin to margin.

The upper layer is composed of an epidermis and a deeper neural layer (neural at least in its apical region). The epiblast is composed of flattened cells that abut against the highest angle of the periblast. In no case does the ring appear to arise by an infolding of this layer; on the contrary, there is sometimes a small space left between the marginal epiblast cell and the nearest cell of the ring (so in 27th). The deeper neural layer, however, bends into the infolding lower layer as shown in the twenty-seventh, thirty-second, thirty-fifth, and thirty-seventh. The nuclei may be seen in all stages of division in the ring region.

The number of cells in a section of the ring varies from two (32nd) to four (27th, 37th). In the floor, the ring is one cell deep (except in apical region — see above) or sometimes two cells deep (most often at the inner edge). Later it is plainly one cell deep.

The *cleavage-cavity* begins with the tenth section and ends with the sixtieth.

The periblast is thicker under the apex than at the opposite point.

Section sixty is much thinner than section five (both are at the boundary of the cleavage-cavity or at a point where the lower layer begins to be distinct from the upper layer).

The periblast plainly does not contribute elements to the ring; it is more difficult to decide about the axial portion. In some sections, notably in the axial portion, I find cells sometimes more or less delimited in the periblast (3rd, 5th, 6th, 8th). This delimitation does not, however, show that periblast cells pass into the cap, as such are visible at *certain stages* of the nuclear transformations. I am inclined to think — not certain — that the periblast does not enter into the ring at any point.

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